

PS Claim 34; Page 56; 71pp; English.

PT New recombinant ribonucleases, used for killing target cells, e.g. for

PT New recombinant ribonucleases, used for killing target cells, e.g. for

PS	Claim 4; Page 59; 71pp: English.
XX	
CC	The present sequence is a recombinant Rana pipiens ribonuclease protein
CC	(RAPRL1) with Met at position 1 attached to (His) ₆ tag and Met24Leu.
CC	Carboxy terminal end of recombinant RAPRL1 has a covalently bound ligand
CC	binding moiety, which can be a LL2 antibody directed against CD22 on
CC	cancerous B cells or human chorionic gonadotropin (hCG) effective
CC	against Kaposi's sarcoma cells. Recombinant ribonucleases can be
CC	expressed in bacteria without an N-terminal methionine due to the
CC	presence of a signal peptide that is cleaved by bacteria. The soluble
CC	expression of ribonuclease allows the proteins to be fused in-frame with
CC	ligand binding moieties to form cytotoxic fusion proteins. They can be
CC	used for treatment of cancer and autoimmune diseases.
XX	
SQ	Sequence 105 AA;
XX	
QY	Query Match 98.3%; Score 566; DB 20; Length 105;
Db	Best Local Similarity 98.1%; Pred. No. 9,8e-62;
Matches 102; Conservative 1; Mismatches 1; Indels 0; Gaps 0;	
QY	1 OQMLFFOKKHLNTRDNDNIMSTNLFHCCKKNFPIRSPREPVKATCGITASKNVLT 60
Db	2 qdwlfcgkhlntctdvcdmllstclflfckckntftysrpepvakicgylasknvlct 61
QY	61 SEFYLSDCNVTSRPCKRYKLRKSTNFCVTCENQAPVHFVGVC 104
Db	62 sefytsdcnvtarspcykylkksntfcvcncgaphvfvgvghc 105
XX	
RESULT 6	
AA128870	AA128870 standard; Protein; 104 AA.
XX	
AC	AA128870;
XX	
DT	25-JAN-2000 (first entry)
XX	
DE	Recombinant RAPRL1 Gln1Ser amino acid sequence.
XX	
KW	Recombinant Rana pipiens ribonuclease; RAPRL1 Gln1Ser; covalently bound;
KW	LL2 antibody; ligand binding moiety; CD22; cancerous B cell; frog;
KW	Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
KW	recombinant ribonuclease; cytotoxic fusion protein; cancer; RNase;
XX	autoimmune disease.
XX	
OS	Rana pipiens.
OS	Synthetic.
XX	
FX	Key Location/Qualifiers
FT	Misc-difference 1 /note= "Wild type Gln replaced with Ser"
FT	
XX	
PN	WO950398-A2.
XX	
PD	07-OCT-1999.
XX	
PP	26-MAR-1999; 99WO-US06641.
XX	
PR	27-MAR-1998; 98US-0079751.
XX	
PA	(USSH) US DEPT HEALTH & HUMAN SERVICES.
XX	
PI	Newton DL, Rybak SM.
XX	
DR	WP1; 1999-610847/52.
DR	N-PSDB; AA208128.
XX	
PT	New recombinant ribonucleases, used for killing target cells, e.g. for
XX	treating cancers, viral infections or autoimmune diseases -
XX	
PS	Claim 34; Page 60; 71pp: English.

CC	The present sequence is a recombinant Rana pipiens ribonuclease (RapLr1)
CC	protein with GlnSer. Carboxy terminal end of recombinant RapLr1 has a
CC	covalently bound ligand binding moiety, which can be a IL2 antibody
CC	directed against CD22 on cancerous B cells or human chorionic
CC	gonadotrophin (hCG) effective against Kaposi's sarcoma cells. Recombinant
CC	ribonucleases can be expressed in bacteria without an N-terminal
CC	methionine due to the presence of a signal peptide that is cleaved by
CC	bacteria. The soluble expression of ribonuclease allows the proteins to
CC	be fused in-frame with ligand binding moieties to form cytotoxic fusion
CC	proteins. They can be used for treatment of cancer and autoimmune
CC	diseases.
XX	
SQ	Sequence 104 AA;
OY	
Db	2 DMLTFKKHLNTRDVCNIIMSTNLFFHKCKNTFTYSRPEVNAICKGIATSKNVLTTS 61 2 dwtlftgkhlntlrctdvcnaimscnlflfckdknftlysrppvnaickgiatsknvlts 61
OY	62 EPIYSDCNWTSRPCKYKILKKTNTFCVTGCENAPRHFGVGHC 104 62 efiysdcnwtsrpkylklkstntfcvtgcenqaprhfvgyghc 104
RESULT	7
ID	AAI28871 standard; Protein: 105 AA.
XX	
AC	AAI28871:
DT	25-JAN-2000 (first entry)
XX	
DE	Recombinant Met(-1) RapLr1 Gln1Ser amino acid sequence.
XX	
KW	Recombinant Met(-1) Rana pipiens ribonuclease Gln1Ser; RapLr1; CD22;
KW	covalently bound; IL2 antibody; ligand binding moiety; cancerous B cell;
KM	Kaposi's sarcoma; human chorionic gonadotropin; hCG; signal peptide;
KW	recombinant ribonuclease; cytotoxic fusion protein; cancer; frog;
KW	autoimmune disease; RNase.
XX	
OS	Rana pipiens.
XX	
OS	Synthetic.
XX	
FH	Key
FT	Misc-difference 1 Location/Qualifiers
FT	/note= "Met not found in wild type RapLr1"
FT	Misc-difference 2 /note= "Wild type Gln replaced with Ser"
XX	
PN	WC9950398-A2.
XX	
PD	07-OCT-1999.
XX	
FE	26-MAR-1999; 99WO-USO6641.
XX	
PR	27-MAR-1998; 98US-0079751.
XX	
PA	(USSH) US DEPT HEALTH & HUMAN SERVICES.
XX	
PI	Newton DL, Rybak SM;
XX	
DR	WPI: 1999-610847/52.
XX	
DR	N-PSDB: AAZ08129.
XX	
XX	New recombinant ribonucleases, used for killing target cells, e.g. for
XX	treating cancers, viral infections or autoimmune diseases -
XX	Claim 34; Page 61; 71pp; English.


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AAB31666
ID AAB31666 standard; protein; 104 AA.
XX
AC AAB31666;
XX
XX
DT 30-APR-2001 (first entry)
XX
DE Amino acid sequence of a frog ribonuclease protein.
XX
XX Frog; ribonuclease; ranpiRNAse; RNase.
XX
OS Rana pipiens.
XX
XX Key Location/Qualifiers
XX FT Modified-site 1
XX FT /note= "this gin is autocyclised to pyroglutamic acid"
XX
PN US6175003-B1.
PD 16-JAN-2001.
XX
XX 10-SEP-1999; 99US-0394268.
XX
XX 10-SEP-1999; 99US-0394268.
XX
XX (ALFA-) ALFACELL CORP.
XX
XX Saxena SK;
XX
XX MPI: 2001-167808/17.
XX
XX New nucleic acids encoding a ribonuclease (Rnase), useful for the
XX precise targeting of Rnase to a predetermined cell receptor
XX
XX Claim 1: Columns 5-6; 7pp; English.
XX
XX The present sequence represents a frog ribonuclease protein (ranpiRNAse)
XX (Rnase). The specification describes a synthetic ribonuclease protein,
XX in which the addition of cysteine in the ribonuclease facilitates the
XX chemical linking of a targeting molecule by the single reactive
XX sulfhydryl group. The specification also describes a method for the
XX production of ranpiRNAse using DNA technology instead of processing
XX biological material. The re-engineering of the protein molecule allows
XX easier attachment to a targeting molecule thereby making it possible for
XX the ribonuclease to be delivered to a particular cell receptor where it
XX might be most effective.
XX
XX Sequence 104 AA:
SQ

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Query Match 95.0%; Score 547; DB 22; Length 104;
Best Local Similarity 95.2%; Pred. No. 2.1e-59;
Matches 99; Conservative 2; Mismatches 3; Indels 0; Gaps 0;

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OY 1 ODMLTFOKKNHNTNRDVCNIIIMSTNLFHCKDKNTFTYSRPEPVKAICGIIASKNVLT 60
DB 1 qdwlrtgkhhltntdvdcnlnstlnfckdkntftysrpepvkalcgylasknvl 60
OY 61 SEFYLSDCNVTSRPCKYKIKLKSTNFCVTCENQAPVHFVGVC 104
DB 61 setylsdcnvtsrpkckykiklstnkfcvtcenqapvhfvgvc 104

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RESULT 11
AAW35126
ID AAW35126 standard; Protein; 379 AA.
XX
XX AAW35126;
XX
XX 20-APR-1998 (first entry)
XX
XX R. pipiens recombinant Rnase ronc fusion protein 2.
XX

```

KW RNase A; ribonuclease; cytotoxic; onconase; nonc; immunofusion;
KW tumour cell growth; frog.
XX
XX Rana pipiens.
XX Synthetic.
XX WO9731116-A2.
XX
XX 28-AUG-1997.
XX
XX 19-FEB-1997; 97WO-US02588.
XX
XX 21-FEB-1996; 96US-0011800.
XX
XX (USSH ) US DEPT HEALTH & HUMAN SERVICES.
XX
XX Bogue L, Newton DL, Rybak SM, Wlodawer A;
XX
XX MPI: 1997-435168/40.
XX
XX N-PSDB; AAT94964.
XX
XX Ribonuclease molecules based on native Onconase - used for killing
XX cells, particularly tumour cells
XX
XX Disclosure; Page 68; 90pp; English.
XX
XX Sequences AAW35125 to AAW35135 represent recombinant fusion proteins
XX (ronc) which are modifications of the Rnase Onconase (RTM) (nonc). Such
XX novel ribonuclease molecules are highly cytotoxic and can be used alone
XX or to form chemical conjugates or to target recombinant immunofusions.
XX They are used particularly for decreasing tumour cell growth. They can
XX also be used for cell separation in vitro by selectively killing unwanted
XX types of cells, e.g. in bone marrow prior to transplantation into a
XX patient undergoing marrow ablation by radiation, or for killing leukaemia
XX cells or T-cells that would cause graft versus host disease. The toxins
XX can also be used to selectively kill unwanted cells in culture. The new
XX ribonucleases have increased cytotoxic activity compared to nonc and
XX also lower immunogenicity in humans.
XX
XX Sequence 379 AA:
SQ

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Query Match 95.0%; Score 547; DB 18; Length 379;
Best Local Similarity 95.2%; Pred. No. 1.1e-58;
Matches 99; Conservative 2; Mismatches 3; Indels 0; Gaps 0;

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OY 1 ODMLTFOKKNHNTNRDVCNIIIMSTNLFHCKDKNTFTYSRPEPVKAICGIIASKNVLT 60
DB 26 qdwlrtgkhhltntdvdcnlnstlnfckdkntftysrpepvkalcgylasknvl 85
OY 61 SEFYLSDCNVTSRPCKYKIKLKSTNFCVTCENQAPVHFVGVC 104
DB 86 setylsdcnvtsrpkckykiklstnkfcvtcenqapvhfvgvc 129

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RESULT 12
AAR12344
ID AAR12344 standard; protein; 104 AA.
XX
XX AAR12344;
XX
XX 08-AUG-1991 (first entry)
XX
XX Protein with activity against cancer cells.
XX Frog eggs; Tamoxifen; Stelazine; cancer.
XX
XX Rana pipiens.
XX
XX WO9107435-A.
XX
XX 30-MAY-1991.
XX

PF 26-OCT-1990; 90WO-US06185.
 XX
 PR 18-MAY-1990; 90US-0526314.
 PR 13-NOV-1989; 89US-0436141.
 XX
 PA (ALFA-) ALFACELL CORP.
 XX
 PI Ardelit WJ, Mikulski SM;
 XX
 DR WPI; 1991-178059/24.
 XX
 PT New protein from fertilised eggs of Rana pipiens - active against
 PT cancer cells, esp. in combination with Tamoxifen or Stelazine
 PT (trifluoro-per-azine).
 XX
 PS Claim 7; Fig 2; 33pp; English.
 XX
 CC The protein is derived from fertilised frog eggs. It has an iso-
 CC electric point of 9.5 - 10.5, a blocked N-terminal gp. and is free
 CC of carbohydrates. It is active against certain cancer cells. The
 CC combination of the protein and (2-1-p-dimethylaminoethoxyphenyl)-1,
 CC 2-diphenyl-1-butene) citrate salt (Tamoxifen) is much more bio-
 CC active than the separate entities against human pancreatic Asp-1
 CC adenocarcinoma, and the combination of protein and (10-(3-(4-methyl
 CC piperazin-1-yl)-propyl)-2-trifluoromethylphenothiazine (Stelazine)
 CC is much more reactive than the separate entities against human lung
 CC A-549 carcinoma. Activity has also been shown against human sub-
 CC maxillary epidermoid carcinoma A-253 cells, human ovarian adeno-
 CC carcinoma NIH-OVCAR-3 cells, human leukaemic HL-60 cells, human
 CC COLO 320 DM cells, human LOX melanoma and human lung squamous car-
 CC cinoma HT-520 cells.
 XX
 SQ Sequence 104 AA;

Query Match 94.4%; Score 544; DB 12; Length 104;
 Best Local Similarity 94.2%; Pred. No. 4.9e-59;
 Matches 98; Conservative 3; Mismatches 3; Indels 0; Gaps 0;

QY 1 ODMLTFOKKHILNTRDVCNIIIMSTNLFHCKDKNTFIYSRPEPVKAICKGIASKNVLT 60
 :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
 Db 1 edwltfgkhhltntdvcdmstnlfhckdkntfysrpepvkaickgiasknvl 60
 QY 61 SEFIISDCNVTSRPCKYKIKKSTNFCVTCENQAPVHFVGVGHC 104
 :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
 Db 61 sefjlsdcnvtsrpckykiksktnkfcvtcengapvhhfvgvsc 104

RESULT 13
 AAR47303
 ID AAR47303 standard; protein; 104 AA.
 XX
 AC AAR47303;
 XX
 DT 09-SEP-1994 (first entry)
 XX
 DE ONCONASE (pharmaceutical protein).
 XX
 KW Oncinase; pharmaceutical; protein; adenocarcinoma; treatment;
 KW cisplatin; melphalan; adriamycin; ovarian cancer; ovary.
 XX
 OS Synthetic.
 XX
 PN WO9403197-A.
 PD 17-FEB-1994.
 XX
 PF 02-JUL-1993; 93WO-US06357.
 XX
 PR 30-JUL-1992; 92US-0921180.
 XX
 PA (ALFA-) ALFACELL CORP.
 XX

PI Ardelit WJ, Mikulski SM;
 XX
 DR WPI; 1994-065396/08.
 XX
 PT Pharmaceutical conty. Cisplatin, Melphalan or Adriamycin - active
 PT in-vitro against OVCAR-3 human ovarian adenocarcinoma cells
 XX
 PS Claim 7; Page 13; 18pp; English.
 XX
 CC This pharmaceutical protein (ONCONASE) is used in the production of
 CC a bioactive pharmaceutical composition also comprising one of
 CC Cisplatin (cis-diaminedichloroplatinum), Melphalan, (4-[bis-(2-
 CC chloroethyl)amino]-L-phenylamine) or Adriamycin (Doxorubicin HCl).
 CC The composition has bioactivity in vitro against OVCAR-3 human
 CC ovarian adenocarcinoma cells.
 XX
 SQ Sequence 104 AA;

Query Match 94.4%; Score 544; DB 15; Length 104;
 Best Local Similarity 94.2%; Pred. No. 4.9e-59;
 Matches 98; Conservative 3; Mismatches 3; Indels 0; Gaps 0;

QY 1 ODMLTFOKKHILNTRDVCNIIIMSTNLFHCKDKNTFIYSRPEPVKAICKGIASKNVLT 60
 :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
 Db 1 edwltfgkhhltntdvcdmstnlfhckdkntfysrpepvkaickgiasknvl 60
 QY 61 SEFIISDCNVTSRPCKYKIKKSTNFCVTCENQAPVHFVGVGHC 104
 :|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
 Db 61 sefjlsdcnvtsrpckykiksktnkfcvtcengapvhhfvgvsc 104

RESULT 14
 AAM00736
 ID AAM00736 standard; protein; 104 AA.
 XX
 AC AAM00736;
 XX
 DT 22-MAY-1997 (first entry)
 XX
 DE Protein derived from frogs eggs.
 XX
 KW Rana pipiens; ovarian adenocarcinoma NIH-OVCAR03 cell; frog; egg;
 KW submaxillary epidermoid carcinoma A-253 cell; tumour; human;
 KW leukaemic HL-60 cell; COLO 320 DM cell; colon adenocarcinoma;
 KW LOX melanoma; lung squamous carcinoma HT-520 cell.
 XX
 OS Rana pipiens.
 XX
 PN US5559212-A.
 PD 24-SEP-1996.
 XX
 PF 06-APR-1988; 88US-0178118.
 XX
 PR 03-FEB-1992; 92US-0814332.
 PR 06-APR-1988; 88US-0178118.
 PR 13-NOV-1989; 89US-0436141.
 PR 01-AUG-1994; 94US-0283970.
 XX
 PA (ALFA-) ALFACELL CORP.
 XX
 PI Ardelit WJ;
 XX
 DR WPI; 1996-442459/44.
 XX
 PT New isolated Rana pipiens frog protein - useful for the treatment of
 PT tumours.
 XX
 PS Claim 1; Column 8; 7pp; English.
 XX
 CC This sequence represents a protein which was prepared by homogenisation
 CC of Rana pipiens frogs eggs. This protein is used for treating tumours

CC In humans. Especially this protein was active against human
CC submaxillary epidermoid carcinoma A-253 cells, human ovarian
CC adenocarcinoma NH-OVCAR3 cells, human leukemic HL-60 cells, human
CC COLO 320 DM cells originally isolated from colon adenocarcinoma, human
CC LOX melanoma and human lung squamous carcinoma HT-520 cells.
XX
SQ Sequence 104 AA:

Query Match 94.4%; Score 544; DB 17; Length 104;
Best Local Similarity 94.2%; Pred. No. 4.9e-59;
Matches 98; Conservative 3; Mismatches 3; Indels 0; Gaps 0;

QY 1 QDWLTFQKKHLNTRVDNCIIMSTNLFHCKDKNTFIYSRPEPVKAICKGIASKNVLT 60
:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db 1 edwltfqkhlnttrvdncimstnlfhckdkntfiysrpepvkaickgiasknvl 60
QY 61 SEFYLSDCNVTSPCKYKLLKSTNFCVTCENQAPVHFGVGHC 104
|||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db 61 sefysdcnvtspckykllkstnfccvtenqapvhfvgvsc 104

RESULT 15

AAW06543
ID AAW06543 standard; protein; 104 AA.

XX AAW06543;

DT 22-AUG-1997 (first entry)

DE Antitumour protein from Rana pipiens oocytes.

KW Tumour; chemotherapy; radiotherapy; frog.

OS Rana pipiens.

PN WO9639428-A1.

PD 12-DEC-1996.

PF 03-JUN-1996; 96WO-US08304.

PR 06-JUN-1995; 95US-0467955.

PA (ALFA-) ALFACELL CORP.

PI Ardelt WJ;

DR WPI: 1997-043063/04.

PT Antitumour proteins from Rana pipiens oocyte(s) - have fewer
PT disadvantages than chemotherapy, surgery and radiotherapy

PS Claim 7; Page 27; 45pp; English.

CC The present sequence is a specifically claimed example of an
CC antitumour protein from the generic protein in AAW18224, with the
CC molecular weight 12000. This is one of two preferred proteins (the
CC other in AAW06544) that have been isolated from Rana pipiens oocytes.
CC Both proteins have a blocked amino terminal group and are essentially
CC free of carbohydrates. The proteins are used to treat tumours. Use of
CC the peptides has fewer disadvantages than chemotherapy, radiotherapy
CC and surgery in the treatment of tumours.

SQ Sequence 104 AA:

Query Match 94.4%; Score 544; DB 18; Length 104;
Best Local Similarity 94.2%; Pred. No. 4.9e-59;
Matches 98; Conservative 3; Mismatches 3; Indels 0; Gaps 0;

QY 1 QDWLTFQKKHLNTRVDNCIIMSTNLFHCKDKNTFIYSRPEPVKAICKGIASKNVLT 60
:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||

Db 1 edwltfqkhlnttrvdncimstnlfhckdkntfiysrpepvkaickgiasknvl 60
QY 61 SEFYLSDCNVTSPCKYKLLKSTNFCVTCENQAPVHFGVGHC 104
|||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||:|||||
Db 61 sefysdcnvtspckykllkstnfccvtenqapvhfvgvsc 104

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Job time: 248 sec